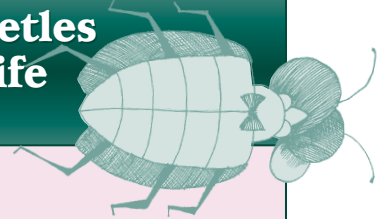


APPENDIX 2

How to Raise and Release *Galerucella* Beetles Outdoors for Controlling Purple Loosestrife in Wisconsin



The Wisconsin DNR's Purple Loosestrife Biological Control Project (WPLBCP) will send you beetles for propagation so you can increase their numbers faster than the beetles can on their own, as well as place them where beetles are needed most. You can rear beetles by ensuring ample food, good living conditions, and no predators, and by carefully choosing release sites. Biological control beetles remain too rare in the state to adequately control purple loosestrife. They are still needed in many more infestations to do the job.

Raising and releasing *Galerucella* beetles is easy and fun. Simply, it includes collecting purple loosestrife roots from your local wetland, potting the plants and growing them in a child-size wading pool, adding beetles when plants are 2 feet tall, and dropping the pots off in your local loosestrife patch about a month later. There are a few crucial details laid out here that will help you succeed in propagating your beetles, yet this is also a project with parts that can be accomplished in many ways. You have the freedom to do it as it suits you—as long as you produce lots of healthy and hungry beetles! Please read this guide carefully, then make the process your own by accomplishing the necessary steps in your own way. If you find an effective and unique way of doing so, please share it with us. Check the Wisconsin DNR's web site annually for updates to this and other appendices.

A PROJECT TIMELINE: GETTING READY

In order to plan ahead, please consider the following typical outdoor rearing timeline, starting in the fall (**Figure A.2.1**). The timeline is based on the biology of the plants and beetles, our temperate climate, and your location, and should result in the healthiest beetles. It is possible to rear beetles indoors earlier in spring and entirely

within the school year. Preliminary research, however, suggests that beetles produced this way are less successful in establishing outdoors. Even if true, all produced beetles have value and the additional educational benefits from more student exposure to the whole rearing process may be worth the tradeoff. More research will be done and, if warranted, a separate indoor rearing appendix will be produced and winter beetles made available.

In the summer before spring rearing you should become familiar with wetland plants to be sure you can correctly identify purple loosestrife. In the fall, search your local wetland for purple loosestrife plants that you want to use



A. HUDSON

Marking purple loosestrife plants with colored flags in the fall helps you locate them in the early spring.





Appendix 2.

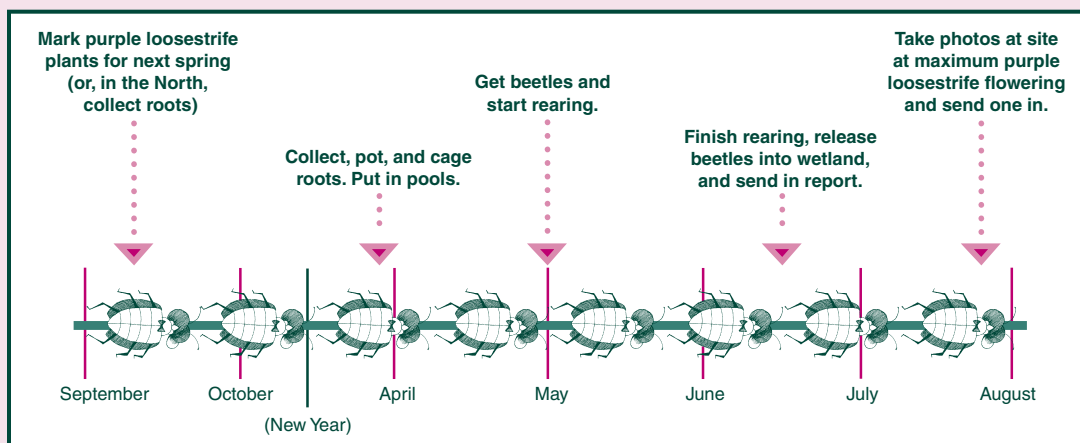


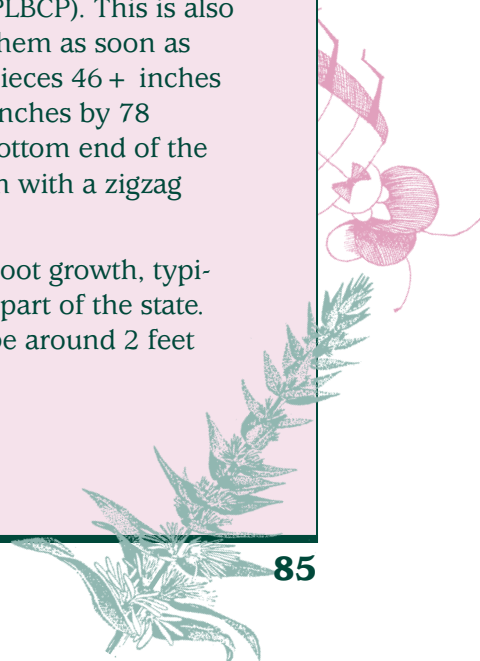
Figure A.2-1. Yearly beetle rearing and maintenance activities.

and mark them with brightly colored plastic flagging. At that time, plant stems and leaves should still be present to help you choose the correct and best plants. If you need to find a site, access the Great Lakes Indian Fish and Wildlife Commission's web site (see Appendix 1 for instructions on using the site) or consult the WPLBCP.

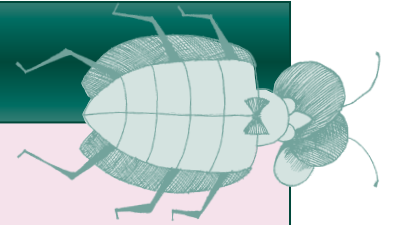
If you live north of Wausau, consider digging your plants in the fall after a few hard frosts, rather than in spring when a late thaw could delay digging. An early start for your plants is important for ensuring that they grow large enough to receive beetles when they become available. Consider buying a wading pool in fall as well, since they are generally less expensive then and often unavailable until late in the spring.

Winter is the time to collect needed tools and materials. Appendix 3 lists everything you might need (some materials may be available free from the WPLBCP). This is also the time to sew your netting into sleeve cages since you will need them as soon as you pot your plants. Cage construction is simple. Start with fabric pieces 46 + inches by 78 inches. Fold each piece along the short side to make it 23 + inches by 78 inches (doubled). Sew down the 78-inch open seam, tapering the bottom end of the cage to match the circumference of your pots. Sew each seam again with a zigzag stitch to strengthen it.

In the spring, dig roots as soon as wetlands thaw and before any shoot growth, typically early April in the South and mid- to late April in the northern part of the state. About 4 weeks later, usually early to late May, your plants need to be around 2 feet tall to receive beetles.



Appendix 2. (continued)



In mid-summer, about 4-8 weeks after adding beetles, you will release your new beetles and send release site information to the WPLBCP (see Appendix 5.) It is very important to take two photographs of your site several weeks later, when the plants are in fullest bloom, and send one photo to the WPLBCP. The other will give you a record of the early site for comparison with photos taken in later years to easily see satisfying changes in the site's vegetation. Monitoring procedures are outlined in Appendix 8.

COLLECTING ROOTS

Purple loosestrife is a state-listed noxious weed. Before cultivating the plant, you must send a copy of your signed permission form/permit (Appendix 4) to the Wisconsin DNR in order to raise plants legally. The only legal purpose for cultivating loosestrife is to produce biological control insects.

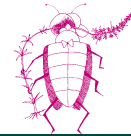
Purple loosestrife is a perennial, with only the above ground plant parts dying each fall. The aim in growing plants from pre-existing roots is to quickly produce the largest plants with the most foliage possible in order to maximize beetle feeding in the spring and summer.

Dig roots *immediately* after your wetland is ice-free. This both prevents damage to tiny new shoots during transplanting and allows you to set up the best conditions for fast early growth of the transplants. If you have a northern Wisconsin site, where roots are often frozen late into spring, consider collecting them the previous fall. You must then store them some place moist and cool over the winter. This may be done outdoors under a shaded tarp or in a water-filled pool, or indoors under moist burlap at 40 degrees or lower and dark, such as in a root cellar. Always dig more roots than you need because many will not survive until spring. If a late spring is delaying your digging, you can travel to a southern location to get roots. You can locate appropriate sites on the Great Lakes Indian Fish and Wildlife Commission's web site (see Appendix 1 for instructions on using the site) or by consulting the WPLBCP.

Starting with large roots is critical, so *choose the largest roots* that will fit into your pots to produce the most foliage possible and avoid premature plant death from larval feeding. Gauge the size of a clump by the number of stems from the previous growing season. A clump with 6-8 stems is probably large enough. Generally, more stems work better. Treat the clump as a single plant, though it is just as likely to be several.

Choose a wetland with good footing and vehicle access since roots are often heavy. Plants in friable soil, sandy soil, or standing water are usually the easiest to dig. Clip the old stems of a clump, leaving a "handle" of about 8 inches. Use a shovel to cut, or a fork to loosen, around the outer base of the clump and lever the roots out by pulling up with your legs and rocking backwards. A large clump can be pulled or cut apart if it is too heavy to carry or too big for a pot, especially if it is more than





Appendix 2.



PHOTOS: B. WOODS

Top: A garden fork can be used to loosen purple loosestrife roots before pulling them up. Note the flagging from the previous autumn.

Bottom: Planting a dozen or more stems per pot will help ensure adequate food for the *Galerucella* beetles.

one plant. Clip the remaining stem stumps and pull off as much soil, other plant roots, organic matter, and dead loosestrife roots (black and brittle, on the bottom) as possible and leave this material in the wetland. Haul roots out of the wetland and transport them in garbage bags or tubs for ease of carrying and to prevent spreading plant parts or seed-contaminated soil. Wear appropriate boots and clothes, including protective eyewear.

Transplant at least 12-15 roots for each 10 growing plants desired. Extra plants will be especially useful for overwintering captive beetles on-site.

POTTING ROOTS

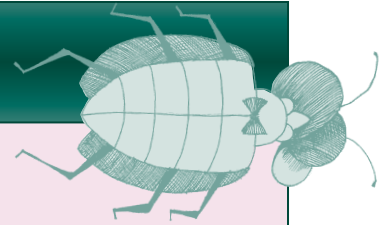
In an empty pool, mix water with your potting soil until it is thoroughly moist. Fill each pot with at least 3 inches of soil, adding more, if necessary, for the crown of each odd-sized root to be at the same height as the others, and a couple inches below the top of its pot. Sprinkle in a teaspoon of slow-release fertilizer (if not already in the soil mix) and mix it with the top inch of soil.

In another empty pool or container, spray-wash each root with a garden hose to remove most of the remaining organic material and mud, especially from the root crown, to get rid of any plant or insect predators and their eggs. Transfer waste material from this process back to the original wetland or to a capped landfill to avoid spreading loosestrife.

Place the largest root mass possible into each pot. If necessary, several small roots can be combined to produce the necessary 6-8 new stems. Clip any jutting root tips to allow packing in more root mass. Pack each pot with soil to within 2 inches of the top, filling all air pockets around the plants' roots. Larvae need to be able to penetrate the soil surface, so do not pack the soil too firmly. Water sparingly at this time to help settle the soil surface. Starting with saturated soil reduces the need to water. Hereafter, always water by filling the pool, not the pots, to prevent washing soil and fertilizer into the pool water.



Appendix 2. (continued)



POOL SET-UP AND PLANT CARE

Place a net sleeve cage over each potted plant *as soon as possible*. This protects the plant from damage and infestation by plant or insect predators. The cage must be securely closed at both ends. Duct tape one end of the cage to the dry pot top, above the water line. (You may use a draw cord or large rubber band to secure the net over the top of the pot, but adding tape will prevent wind from blowing the net off.) Try to leave as much fabric above the tape as possible to give the plant maximum amount of room for growth. Tie the top of the cage closed with a cord or wire, place the pot into a pool and suspend the bag from a support. The net cages must be supported to allow plants to grow unhindered. Many kinds of support systems will work (Figure A.2.2).

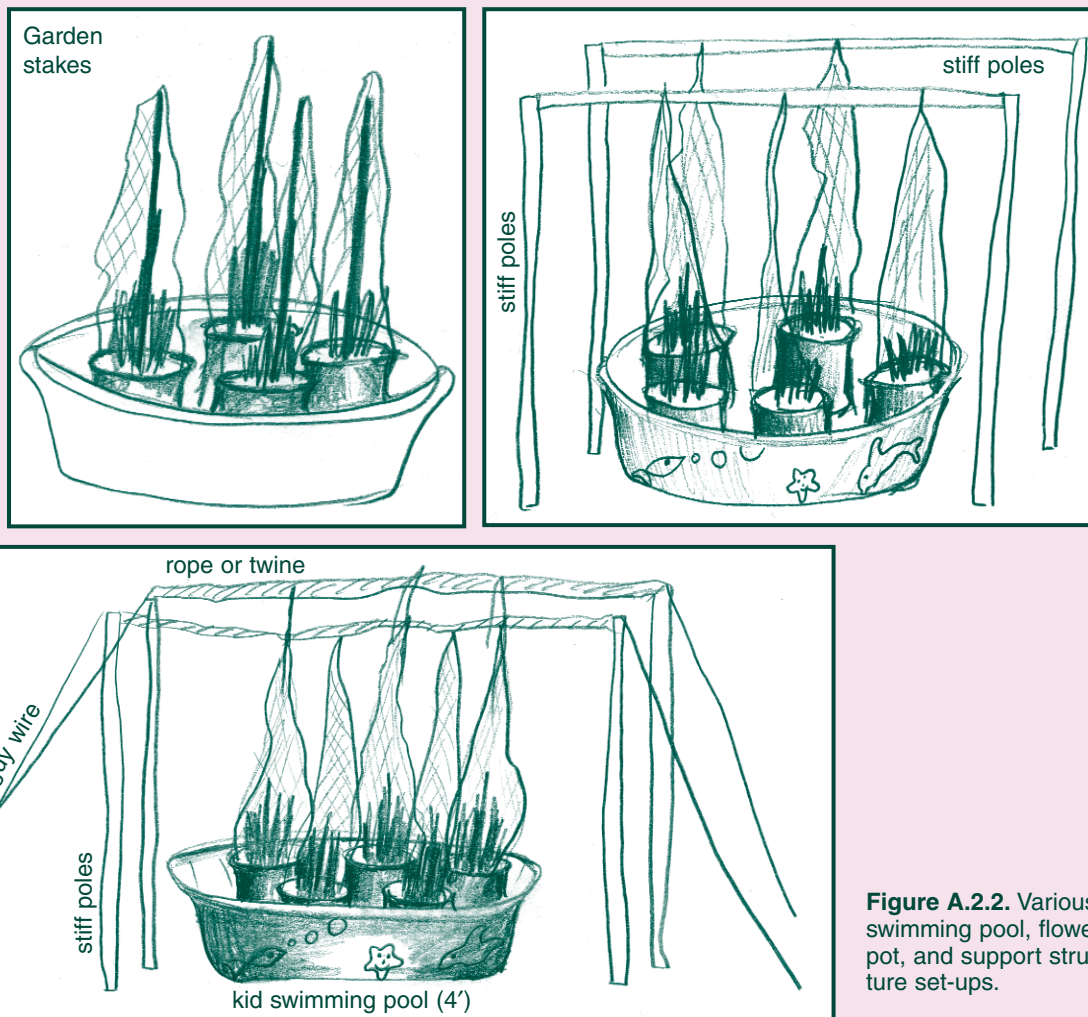


Figure A.2.2. Various swimming pool, flower-pot, and support structure set-ups.





Appendix 2.

These range from a thin post, such as conduit, inside of each cage to exterior post systems with suspended cross members. (Interior supports should only be used where wind is minimal since they often allow pots to blow over). Guy lines can give added stability to any system.

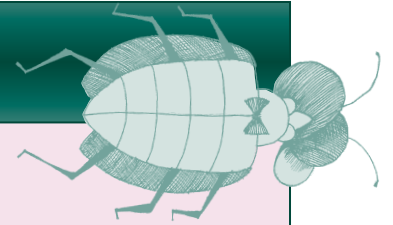
Place pools in full sunlight, but out of strong winds. Arrange pots around the perimeter of a pool to give each plant maximum sunlight. Five to six pots fit around a 4-foot pool with extras in the center. Two of these pool set-ups are the best option for rearing 100 beetles. They are also easier to transport and offer more versatility in space arrangements. Ten pots also fit around a 5-foot pool with ample center room for extras, but



PHOTOS: B. WOODS

Cooperators use a variety of materials, techniques, and set-ups for rearing *Galerucella* beetles.

Appendix 2. (continued)



less sunlight may reach each plant than with 4-foot pools. At least one plant in a project should be cage-less to attract any escaped beetles. Other extra plants should be caged as replacements for weak plants.

Always keep several inches of water in the pools containing the plants. Check the pools daily, even on weekends, if the weather is hot and sunny. Drill drain holes several inches up a pool's sides, if necessary, to ensure that the maximum water level is never higher than 2-3 inches below soil heights, since pupating beetles do poorly in saturated soils.

You can eliminate mosquito larvae by periodically draining or flushing pools, or by putting in a few native mud minnows or goldfish, both of which survive in low oxygen environments. Frogs may appear; they may also eat mosquito larvae. You may have to replenish any of these critters if water levels drop too low or local predators such as raccoons eat them. Do not let non-native goldfish loose into the wild.

Plants need about 4-6 weeks, depending on weather conditions, to grow 2 feet tall and be large enough for beetle introductions. You may grow them indoors to shorten this time, but move them outdoors before adding beetles. Crowns take 1-2 weeks after potting to begin to grow, but then grow quickly. When stems are 12-15 inches tall, spread the small leaves at the tip of each growing point and carefully pinch it off. This stimulates the growth of lateral stems, provides more foliage, and helps keep plants from growing too tall for their cages.

RAISING YOUR BEETLES

When around 2-feet tall, plants are ready for beetles. Let the WPLBCP know ahead of time when you *expect* this to be and the program will send your beetles on or soon after that date. (There is a small, tax deductible donation to pay for delivery that should be sent to the WPLBCP with your application; see Appendix 4.) Placing beetles on plants that are too small or have too few stems or too little foliage can result in reduced beetle production and even premature death of the plant. This situation requires early beetle release.

Beetles will come to you either shipped overnight from Madison or delivered in-person by Wisconsin DNR or UW-Extension staff. Once you receive them, put the beetles into

Cooperators receive about 100 beetles for rearing and release. Beetles are mailed or delivered by WPLBCP staff.



B. WOODS





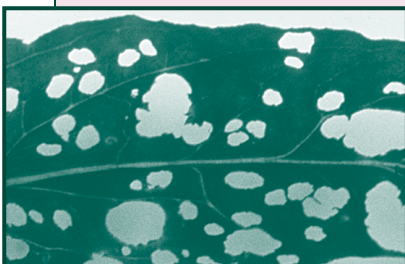
Appendix 2.



L. REGNI



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CORNELL UNIVERSITY

Top: Using an aspirator or similar device makes moving the beetles easy and prevents you from harming them.

Center: Cooperators begin their rearing efforts with adult *Galerucella* beetles.

Bottom: Adult *Galerucella* beetles "skeletonize" leaves.

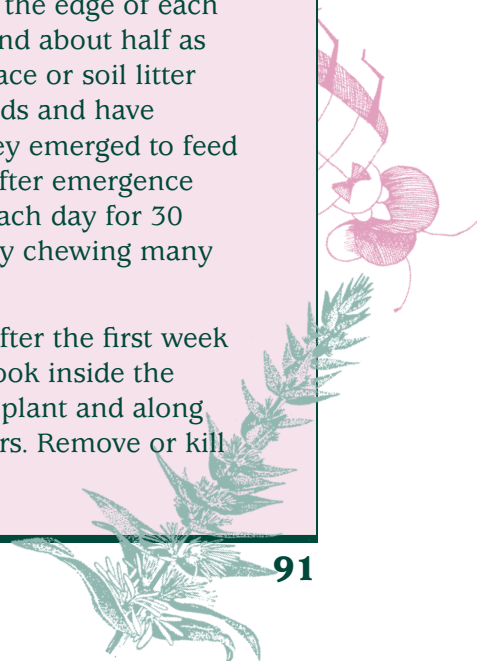
your cages as soon as possible. If you must delay, keep the beetles cool and out of direct sunlight, especially if they remain in the shipping container or the day is hot. If the delay is overnight, open the shipping box and bag inside to give the beetles fresh air. Gently knock the beetles away from the bag opening first since they will be eager to escape. Keep the shipping container, unless instructed to return it.

When you are ready to transfer beetles to your cages, use an aspirator or similar device to move 10 healthy beetles to each plant. If there is any reason to suspect spiders or other predators might be on your plants (e.g., your plants were not netted immediately after transplanting), check them over carefully right before adding the beetles. Open the top of each sleeve cage and simply drop the beetles in. Tightly cinch the tops closed and re-suspend the cages. Avoid using fingers or tweezers to handle the insects. Divide any leftover beetles among your larger plants. As soon as beetles are on your plants it is time to decide where your new beetles will be released.

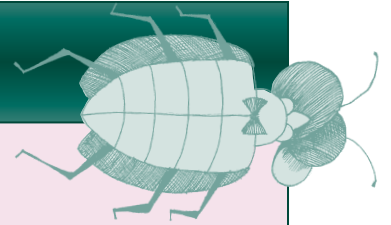
You will see your beetles' four life stages if you watch carefully over the following 4-8 weeks. Many web sites listed in Appendix 9 have color photos that will aid you in identifying them. Note that temperature and weather conditions are important factors in the beetles' activity level and growth rate.

Old adults that you receive are from the previous year and are dark brown with a black stripe along the edge of each wing cover. They are 4-6 mm in length and about half as wide. They over-wintered at the soil surface or soil litter either in a wetland or surrounding uplands and have recently been collected in the field as they emerged to feed and mate. They will live about 6 weeks after emergence and each female will lay about 10 eggs each day for 30 days. Adult beetles "skeletonize" leaves by chewing many small, fairly round holes in them.

If *no* adult beetles appear to be present after the first week (also indicated by lack of leaf damage), look inside the cage, especially in the lower parts of the plant and along the soil for live adults and insect predators. Remove or kill



Appendix 2. (continued)



any of the latter. If neither is found, check the cage for holes or other possible means of escape. Check your cage-less plant for escapees and return them, once any means of escape has been fixed.

Eggs are tiny (less than 1 mm) and cream colored, with an uneven black line of frass (insect excrement) deposited on them. They are usually laid in bunches, often along the edge of adult feeding damage on both stems and leaves or in leaf axils. Humidity is important for egg hatching so make sure pools always have water in them to keep humidity high. Eggs hatch 2-3 weeks after they are laid. If you see no eggs, but adults are present, you may have a poor mix of beetle species (there are two) or genders. Try mixing with adults from a cage with lots of eggs.

Larvae are very small (about 1 mm) and hard to see when newly hatched. Larval damage in the shoot tips, called “tip-feeding,” should be obvious early on, especially when accompanied by frass. Larvae are yellow with a dark head capsule and molt five times, each time increasing in size. Over 80% of larval growth occurs in the 4th and 5th larval instars. Their feeding damage is described as “window paning” because the leaf tissue is left brown, thin, and translucent, unlike the holes left by adults. After 2-3 weeks of feeding, large 5th instar larvae move to the soil to pupate. If any plant turns completely brown while larvae are still visible, place that plant into the field immediately and check your other plants daily for the same problem, especially any that are smaller or had many eggs.

Pupae are the transformation stage between larvae and new adults. When most larvae seem to have disappeared, they are likely in the top inch of soil as pupae. During the pupation period, do not allow the upper layers of soil in your pots to be saturated with water for very long or many pupae will not survive. Some pupae manage to end up floating in the pool water; carefully retrieve and return them to the soil in a cage. After 2-3 weeks as pupae, teneral adults emerge from the soil.

New adults (tenerals) will emerge from the soil up to 100 times more numerous than the number of old adults started with 6-8 weeks earlier. They are usually light tan with no dark coloration on their wing covers and are easy to tell apart from old adults, with whom there may be a very slight over-lap in time. They collect at the top of each cage trying to disperse, especially on sunny days. Numbers are low at first, but hundreds more may appear every day, so *place a pot in the field as soon as any are seen in its cage*. Teneral adults will not survive long if plants have turned brown from larval or other adult feeding. In this case, add fresh loosestrife stems (inserted into florist clips with water) to the cages to feed them for a brief time, but release the beetles as soon as possible.





Appendix 2.



B. WOODS

Cooperators transport their potted purple loosestrife plants and *Galerucella* beetles to suitable wetland sites.

RELEASING BEETLES AND FOLLOW-UP

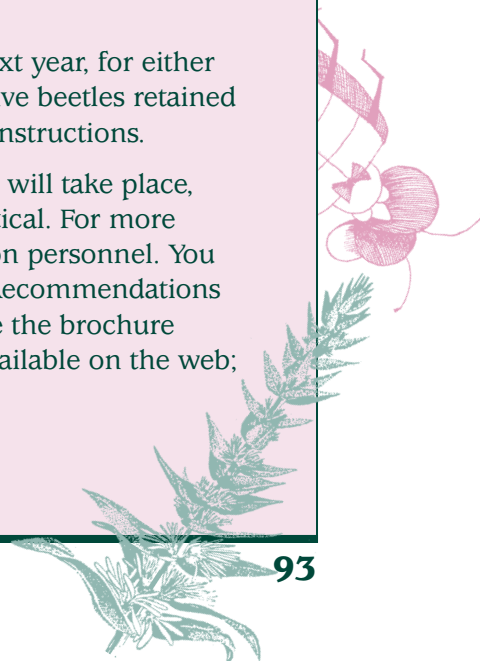
Release your beetles when the *first* new adults appear in your net cages, but decide on suitable release sites much earlier. Ten old adults produce up to 1,000 teneral adults per pot, for a total of 8-10 thousand tenerals when you start with 100 old adults.

This is enough to start 4-5 small colonies, since you should put out a minimum of 2,000 beetles

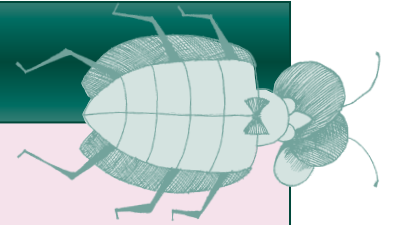
(usually 2-4 pots) on a site to start a viable population there. However, the best strategy the first year is often to set up a couple insectories, which are small, high quality sites for producing new local propagation stock within 2-3 years. Such sites have 50+ healthy, mature loosestrife plants, but are ½ acre or less in size and are well separated from larger loosestrife infestations. Beetle numbers can build up quickly and stay concentrated on such sites, making it both easy to collect breeding stock as well as see great results! These sites should also have landowner assurance of site security, good access and footing, be free from insecticide spraying (e.g., for mosquito control) for at least a year, have only very short or no summer flooding, and be places from which the loosestrife is unlikely to infest new wetlands.

If you wish to try producing your own propagation stock for the next year, for either spring or winter (indoor) rearing, you will need to over-winter captive beetles retained from your first year's rearing. Appendix 7 includes over-wintering instructions.

After setting up insectory sites the first year, or if no further rearing will take place, you can place beetles on any site where no other controls are practical. For more information on site choice, consult Wisconsin DNR or UW-Extension personnel. You can also refer to the "Wisconsin DNR's Purple Loosestrife Control Recommendations and Traditional Methods" in Appendix 6 and other publications like the brochure "Purple Loosestrife: What You Should Know, What You Can Do" (available on the web; see Appendix 9).



Appendix 2. *(continued)*



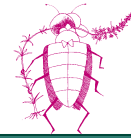
Clustering 2-3 pots around healthy, wild purple loosestrife plants gives the newly reared beetles “field” food and encourages their colonization of the wetland.

Transport potted plants and cages together. Ensure that beetles arrive in good condition by avoiding jarring (as in the back of a pick-up truck) and high temperatures. Fold plants and cages over gently if space is small, such as inside a vehicle.

Cluster pots within several meters of each other in a wetland with each pot adjacent to a large, healthy purple loosestrife plant. Remove the cages and shake out any adults onto nearby foliage. Bend the adjacent loosestrife plant stems into the spent, potted plant stems to allow any remaining larvae and new beetles to walk onto fresh foliage for immediate feeding. Mark either the ends of the site or the individual pot locations with PVC poles or flagging so you can find them again. Wait at least 4 weeks after the release before recovering your pots to allow the remaining beetles to emerge and disperse, or leave the pots until the spring.

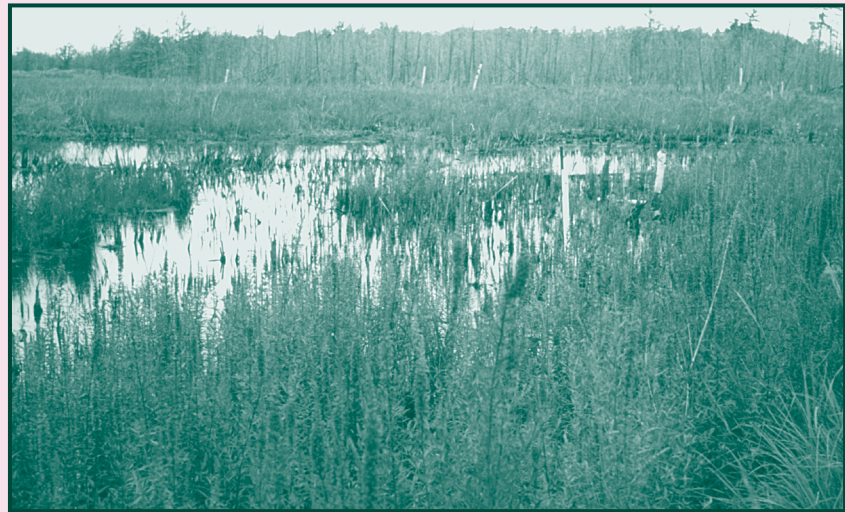
If your beetle release is late, hundreds of beetles may congregate in your cage tops. If no green plant tissue remains, you must release them immediately or feed them, or they will die from the stress of starvation. A different release procedure is recommended to encourage these beetles to stay on the site after release. At the field site loosen the cage bottom from a pot, lift the pot and spread open one side of the cage. Insert several healthy field stems into the cage as you lower it and its pot to the ground. Snug the bottom of the cage around the pot and new stems as well as possible. This gives the new adults additional “field” food, getting them used to the site, before they are actually released. Remove the cages within 2-3 days, but leave the pots as above.





Appendix 2.

Taking photographs during peak loosestrife flowering allows you to document the effect of the beetles on purple loosestrife plants.



B. PRUKA

On the day of every release, please fill out and mail in a copy of the “Insect Release Field Report” in Appendix 5 for each release site. Return to each release site and take two photographs of it in late summer when the loosestrife flowering there is its most spectacular. Send one of your photos to the WPLBCP. This visual record in year 0 can be used to contrast with photographs of, or visits to, the same site in ensuing years to see how much the beetles are affecting the loosestrife. Larvae demolish flower buds and as their numbers build there should be less and less purple on the site at this time of year. Put a marker on the place you take the picture so later shots can be taken from the same spot. This is the easiest way for you to gauge your success with biological control.

New adults feed on leaves for a few weeks, but disappear around mid-August to over-winter in the leaf litter near host plants or in surrounding uplands. They and their feeding damage are often difficult to find then, but look carefully when you return to collect pots or take photographs. Finding beetles is easiest in the spring. Consider visiting your site then, especially as part of optional monitoring procedures (see Appendix 8).

If you have any questions, comments, or suggestions about the beetle rearing process or want to send in site or release information and photographs, please write the Wisconsin Purple Loosestrife Biological Control Project at Wisconsin DNR Research Center, 1350 Femrite Dr., Monona, WI 53716 or call 608-221-6349.

Good luck!

